ORIGINAL ARTICLE

Investigation of Heavy Metals, Nitrate and Nitrite in the Common Available Commercial Packed Drinking Water in Mashhad, Iran

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Abstract

Background: Due to the increasing consumption of bottled water in recent years it is essential to ensure safety measures. To this end, in this study, we aimed to evaluate the concentration of 6 toxic heavy metals, nitrate and nitrite components in the commonly sold bottled water in Mashhad, Iran.
Methods: The 11 best-selling bottled water brands in Mashhad were identified. Eight bottles from each brand were randomly collected and delivered at the same day to toxicology laboratory of Imam Reza Hospital and refrigerated at 4-6 °C. Spectrophotometry and atomic absorption spectrometry were used to measure the nitrate, nitrite, and heavy metals, respectively. The results were analyzed by SPSS version 16 and compared with the WHO and Australian guidelines. Also, the discrepancy between the measured components and the depicted labels' values were compared.
Results: The mean and SD of concentrations of the heavy metals in 11 brands were as below: lead 1.62±0.86 [µg/L], chromium 1.03±0.84 [µg/L], cadmium 0.17±0.07 [µg/L], mercury 3.86±1.57 [µg/L], arsenic 0.89±0.46 [µg/L], aluminum 6.56±4.54 [µg/L]. The mean and SD measured quantities of nitrate, nitrite, and pH were 9.96±5.95 [mg/L], 0.01±0.03 [mg/L] and 7.92±5.95, respectively. There was a significant difference between the label values and the quantitative levels except for 3 brands, which was observed with a p value of 0.518 and 0.642 for nitrate level in N4 and N11 brands, as well as 0.681 for pH level in N7 brand. The measured values of heavy metals, nitrate, and nitrite in all samples were within domestic, WHO and Australian limits, except for mercury in 9 samples which exceeded the Australian standard [less than 1 µg/L].
Conclusion: The heavy metals, nitrate, and nitrite concentrations in all samples were within the domestic, WHO and Australian ranges, except for mercury in 9 samples that exceeded the Australian standard. There was a discrepancy between the entries of the bottled labels and the measured quantities.

Keywords: Nitrate, nitrite, heavy metals, toxic, bottled water

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INTRODUCTION

Globally, the mineral water market has witnessed a substantial growth in recent years. The primary driving factors behind this expansion are increasing concerns about hygiene and health, which make bottled water a more attractive choice compared to tap water. Furthermore, efficient marketing strategies have played a key role in the growing demand for bottled water [1].. Many chemicals may be occurred in drinking water; however, some of them are toxic and thus should be monitored. Having prioritized monitoring and health-giving properties of drinking water's chemical pollutants, it is of great concern to prevent arbitrary misuse of scarce resources [2]. Nowadays, even with the existence of the clean water supplies in every home in urban areas, people mostly prefer to consume bottled drinking water in developing countries, either locally bottled or imported ones. People have many reasons to actually prefer bottled water to tap water. Firstly, undesirable taste of local tap water or even an unpleasant appearance -in case of chlorination and transfer pipes- is an issue. Secondly, they like the convenience of a portable

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bottle [3-5].

The holy city of Mashhad is the second largest city of Iran, situated in the north-east of the country, welcomes in excess of 20,000,000 pilgrims annually from all across the Islamic world. The bottled water is one of the main sources that provide roughly a majority of consumed potable and drinking water in Khorasan Razavi.

National and international guidelines have been made concerning water pollution. Among water quality standards, this study has focused especially on heavy metals, nitrate, and nitrite to ensure that water quality is protected.

The harmful health effects of heavy metals such as lead, mercury, cadmium, chromium, arsenic, and aluminum are of inevitable consequences due to the exposure to these environmental pollutants in drinking water. Some of these effects may damage the nervous system and brain and lead to kidney dysfunction and cancer [6].

Arsenic is one of the human carcinogens, which have been issued by the International Agency for Research on Cancer [IARC] since 1980 [7]. Many research studies have ascertained the association of some cancers with arsenic exposure, namely bladder cancer [8], skin cancer [9], and lung cancer [10]. Additionally, there are some non-carcinogenic effects of chronic arsenic exposure [5, 11]. Neurobehavioral effects are observed with long term exposure to high amounts of arsenic which can end in behavioral changes in later life according to a survey conducted by Tsai et al. [12]. Cadmium is known to be the cause of deterioration of organs such as the kidneys, liver, and lungs due to its long-term exposure [13]. Furthermore, destructive effects on central nervous system, immune systems, and fertility disorder beside a variety of cancers would be stemmed from the exposure to high amounts of cadmium [14, 15]. Impairment of psychological and neurobehavioral functions have also been found after long-term lead exposure having more hazardous effects on children since they are more vulnerable. Although elemental mercury is relatively innocuous, but the toxic effects of inorganic mercury compounds are seen mainly in the kidney with high amounts which does not comply with the mandated standards for drinking water guidelines [6, 16, 17]. Aluminum has been found to be associated with Alzheimer's disease and two severe neurodegenerative diseases, namely Parkinsonism dementia [PD] and amyotrophic lateral sclerosis [ALS] [16, 18, 19]. Circumstantially, nitrate and nitrite, being absorbed from gastrointestinal tract can react directly to hemoglobin and eventually cause methemoglobinemia, which consequently produce oxygenation failure [20, 21]

The present study, therefore, was aimed to conduct a survey of the toxic chemical quantities of 11 different bottled brands of drinking water, which have been randomly selected and purchased in retail outlets in Mashhad, including the information on their physicochemical properties, namely pH, nitrates, nitrates, and selected heavy metals.

MATERIALS & METHODS

Many analytical techniques have been proposed for the measurement of concentrations of heavy metals in water samples, including spectrophotometry [19], ion chromatography [20], atomic absorption spectrometry [21, 22], inductively coupled plasma atomic emission spectrometry [ICP-AES] [23, 24], and near-infrared spectroscopy [22]. We have used different techniques of atomic absorption spectrometry in the toxicology laboratory of the center.

Sample Preparation:

A total of 88 samples were collected from 11 famous brands of drinking bottled water across the town. All samples were subsequently refrigerated at 4-6˚C. They were all delivered to the designated laboratory on the same day for determination of heavy metals, nitrate, nitrite, and pH. Nitrite and nitrate measurements carried out on the day of collection whereas the toxic metals determinations undertook within fortnight.

Sample Analysis:

Atomic absorption spectrometer [Perkin Elmer model 3030 USA] was used for measuring heavy metals during July-September of 2011. Lead, chromium, cadmium, and aluminum were measured by graphite furnace system, but mercury and arsenic concentrations were determined by mercuric-hydride system. The reliability of the method was evaluated by spiking selected metals with five samples and determed recovery, detection limit and accuracy parameters. The accuracy of determination of aluminum, arsenic, cadmium, chromium, mercury, and lead were 97.5%, 98.2%, 99.2%, 99.0%, 98.4% and 99.4%, respectively. UV-Vis spectrophotometer [model DR 5000] was used to measure nitrate and nitrate.

The mean quantities of each measured parameters were compared with the WHO guidelines for drinking water standards and Australian guideline [48, 49]. Based on health considerations complying WHO guideline, the concentration of lead, mercury, aluminum, cadmium, chromium, arsenic, nitrate, and nitrite in drinking water should not exceed 10 µg/L, 6 µg/L, 200 µg/L, 3 µg/L, 50 µg/L and 10 µg/L, 50 mg/L and 3 mg/L, respectively. These parameters are similar according to Australian guideline, with the exception of aluminum, mercury and cadmium, which are less than100 µg/L, 1 µg/L and 2µg/L, respectively.

Data were analyzed by SPSS® version 16 for Windows. Results are presented as mean ±SD for every single brand and eventually for the whole of samples. Furthermore, the labeled data of each brand were compared with measured components by means of one sample T-test and a P value of less than 0.05, considered statistically significant.

RESULTS

Al, Cr, As, Cd, Pb, Hg, nitrate, and nitrite concentrations of all samples were compared with the international standard limits as presented in Table 1. All the measured toxic heavy metals quantities, nitrate, and nitrite in all samples were within the WHO and Australian ranges, except for mercury in 9 samples that exceeded the Australian standard [less than 1 µg/L]. The mean and SD concentrations of all the analyzed samples were depicted in

Table 1. Also, the data of related constituents were collected and depicted in table 2. Regarding the measured nitrate and pH levels, none of the brands precisely matched their label claims, except for three. In these brands, the measurements were in accordance with the label claims, with p-values of 0.518 and 0.642 for nitrate levels in brands N4 and N11, respectively, and a p-value of 0.681 for the pH level in brand N7..

The figures of highest and lowest concentrations of lead were found in N4 [3.15 µg/L] and N2 [0.18 µg/L]. Mercury concentrations were within the normal expected limits in all the samples according to WHO guideline but in comparison with the Australian guideline [Hg <1 µg/L], only 2 samples were below the mandated levels [N.1 and N.11]. The highest

Table 1. Mean and standard deviation concentrations of the toxic chemicals in different brands of bottled drinking water in Mashhad, Iran comparing with WHO and Australian guidelines

	pH	Aluminum [µg/L]	Nitrate [mg/L]	Nitrite [mg/L]	Mercury [µg/L]	Cadmium [µg/L]	Chromium [µg/L]	Lead [µg/L]	Arsenic [µg/L]
N.1	8.08±0.03	3.66±0.06	15. 87±0.07	0.002±0.004	0. 88±0.02	0. 18±0.008	2.76±0.05	0.91±0.04	0.50±0.02
N.2	7.90±0.00	7.92±0.07	6. 85±0.05	0.004±0.002	4.54±0.08	0. 12±0.007	0.86±0.03	0.21±0.02	1.08±0.03
N.3	7.77±0.07	12.80±0.09	15. 12±0.12	0.000±0.001	5.74±0.05	0. 06±0.028	2.29±0.02	2.52±0.05	1.39±0.05
N.4	8.09±0.01	12.60±0.11	3.07±0.15	0.003±0.002	3.52±0.04	0.16±0.013	1.24±0.03	3.03±0.08	0.87±0.03
N.5	7.82±0.12	13.61±0.05	1.68±0.40	0.005±0.001	2.75±0.05	0.14±0.007	0.29±0.03	1.92±0.08	0.22±0.02
N.6	7.98±0.04	2.51±0.38	12.47±0.07	0.009±0.001	1.89±0.06	0. 10±0.008	0.79±0.02	0.51±0.02	0.11±0.004
N.7	7.78±0.03	6.26±0.07	7.77±0.07	0.004±0.001	5.05±0.08	0.18±0.008	1.05±0.34	1.17±0.03	1.12±0.04
N.8	8.09±0.04	2.28±0.48	4.92±0.07	0.001±0.0005	5.80±0.04	0.28±0.014	0.10±0.01	1.75±0.03	1.45±0.03
N.9	7.80±0.00	1.41±0.03	12.15±0.11	0.0005±0.00007	4.88±0.04	0.30±0.0015	0.71±0.02	2.32±0.04	1.38±0.03
N.10	7.89±0.07	3.03±0.04	20.70±0.60	0.001±0.001	3.52±0.03	0.23±0.074	0.18±0.02	1.89±0.02	0.76±0.03
N.11	7.80±0.06	1.12±0.03	1.36±0.25	0.004±0.001	0.92±0.02	0.08±0.016	0.73±0.02	0.96±0.02	0.34±0.02
All analyzed samples	7.92±5.95	6.56±4.54	9.96±5.95	0.01±0.03	3.86±1.57	0.17±0.07	1.03±0.84	1.62±0.86	0.89±0.46
WHO criteria	6.5-8.5	<100	<50	<3	<6	<3	<50	<10	<10
Australian guideline	6.5-8.5	<200	<50	<3	<1	<2	<50	<10	<10

Table 2. Labeled and measured parameters in packaged bottles

Samples	Nitrate Levels on the labels	The mean & SD concentration of Nitrate in the samples	P value for nitrate	pH levels on the labels	The mean & SD of pH levels in the samples	P value for pH
N 1	7.4	15. 87±0.07	0.0	7.7	8.08±0.03	0.0
N 2	-	6. 85±0.05	-	7.8	7.90±0.00	0.0
N 3	4.04	15. 12±0.12	0.0	7.5	7.77±0.07	0.0
N 4	3.5	3.07±0.15	0.518	7.4	8.09±0.01	0.0
N 5	2.4	1.68±0.40	0.002	7.6	7.82±0.12	0.0
N 6	6.4	12.47±0.07	0.003	7.8	7.98±0.04	0.0
N 7	-	7.77±0.07	-	7.8	7.78±0.03	0.681
N 8	0.5	4.92±0.07	0.0	7.4	8.09±0.04	0.0
N 9	1.8	12.15±0.11	0.0	7.2	7.80±0.00	0.0
N 10	2	20.70±0.60	0.0	7.29	7.89±0.07	0.0
N 11	2.4	1.36±0.25	0.642	7.2	7.80±0.06	0.0

concentration of mercury was measured in N.8 with 5.87µg/L. All the samples complied with the permissible range of pH content according to both WHO and Australian guideline.

DISCUSSION

Atomic absorption spectrometry (AAS) is a widely used technique for the determination of heavy metal concentrations in water samples. Several studies have employed AAS to determine heavy metal concentration in water. In a study by Nalatambi in Bandar Sunway, Malaysia, AAS was used to detect the concentrations of zinc, cadmium, chromium, lead, magnesium, calcium, and copper in tap water samples. The results were compared to WHO and EPA guidelines for drinking water quality [44]. Elhamili et. al, in Tripoli, Libya used AAS to estimate the levels of zinc, cadmium, copper, lead and iron in tap and underground water samples. The results were compared to WHO and Libyan standards [45]. Moreover, a study by Idris et. al, in Yobe, Nigeria, used AAS to examine heavy metal concentrations in water samples from a gypsum mining site [46].

Studies highlight several key advantages of AAS for heavy metal analysis in water as follows [44-46]: high sensitivity and selectivity for detecting trace metal concentrations, ability to analyze a wide range of metals including Ca, Cu, Mg, Mn, Zn etc., relatively simple sample preparation involving digestion and dilution, comparison of results to established regulatory standards for water quality.

The results demonstrate that while AAS is a well-established and sensitive technique, other methods like ICP-AES and ICP-MS offer advantages in terms of broader elemental coverage, higher sample throughput, better tolerance of complex matrices, and lower detection limits. The choice of analytical technique depends on the specific requirements of the analysis [47].

Mercury and Aluminum quantities and the alleged health hazards were investigated by Allen et al. in 1989, which assessed 37 brands of domestic and imported mineral waters, 24 of which had one or more components that were not in compliance with the drinking water standards in the United States. They found out mercury in one sample far exceeded the WHO guideline [305 µg/L]. Also aluminum was more than the standard guidelines in 4 samples. Our study was roughly in consistent with that of Allen survey [23], while 9 of our brands exceeded the Australian guidelines for aluminum concentration, all the samples complied with the WHO guideline. These data are similar to the reports in Italy Cicchella et al. 2010 [24] and Barroso et al in 2009 [25] which also reported mercury within the normal ranges in their assessment.

In much the same way as our study regarding the Australian guideline, Ikem et al. [2002] measured much higher Hg concentrations in USA water samples, of up to 79µg/L [26]. Similarly, aluminum content in bottled water exceeded WHO guideline in survey by Krachler et al. [2008], which conducted on 132 brands of bottled water from 28 countries [27]. In the study by Espejo-Herreraa et al. in 2010, most frequently consumed bottled water brands [9 brands] were collected across 11 provinces in Spain, assessing the quantities of nitrate, arsenic, nickel, chromium, cadmium, lead, selenium, and zinc. Concentration range for nitrate [2.3-15.6mg/L], which was of normal determined standard range, is the same as the present study, although, other trace elements level were low and mainly unquantifiable in bottled water [28, 29]

The selected toxic chemicals in other international studies were evaluated and compared with our results as described in Table 3. Our results were also consistent with Bakirrdere et al. [2013] and Ristic et al. [2011] reports regarding lead, cadmium, and arsenic of 17 bottled water samples, which were shown to be within the normal ranges by the WHO [28, 30]. The concentrations of nitrate, lead, and cadmium in measured water samples were of higher figures in this study than that of Azlan et al. 2012, whereas our arsenic rates were lower in its quantity [31]. Guler et al. 2009 also reported that arsenic concentration in one sample was almost three times higher than WHO guideline. In another report, the toxic heavy metal levels were found to be of the normal ranges in their survey, conducted on 70 bottled water samples in Turkey [32]. All the measured constituents in 25 brands of commercially available bottled water in Pakistan, analyzed by Saeed et al. [2009] conformed with the WHO guidelines/directives except for arsenic which exceeded those guideline in one samples in contrast to our study which all the measured arsenic met permissible WHO standards [33]. Ali and colleagues found that nitrate concentrations in bottled drinking water samples from 11 different brands ranged from supra-detection limits to 37 mg/L. Notably, all the brands were within the maximum allowed limit recommended by the WHO [34]. In a separate study, Astel et al. analyzed 47 bottled water brands in Poland, revealing median arsenic and lead values of less than 0.5 mcg/L. Additionally, the water samples did not contain any detectable levels of cadmium [35].

Daniele et al. conducted a study evaluating 10 mineral bottled waters in Chile. Most samples in the study had concentrations below 8 ppm, except for the Cachantun sample, which was nearly five times higher than the other samples [44.3 ppm], but still within the WHO allowance range. The Puyehue [18.97g/l], Jahuel [12.54g/l], and Jumbo [12.76g/l] samples contained between 25% and [33] 90% more than the WHO limit for drinking water. Although cadmium and lead levels in Chile's drinking and mineral water were higher than those set by the EPA [2009] or WHO [2011], all samples taken by the Chilean researchers met internationally recognized standards for these elements. Furthermore, all samples complied with WHO standards for aluminum and chromium [36].

Another study by Naddeo V et al. was conducted across Italy to assess organic and inorganic compounds, which revealed similar figures in comparison with our results. Yet, it reported substantially higher levels of lead [3500 µg/L] and arsenic as well as lower quantities of chromium [37]. Also, in comparison with our study, Misund A et al. and Pip E reported higher level of lead concentration in some

Table 3. Comparison of data from present study with other studies in correlative parameters

Country		Nitrate [mg/L]	Lead [micg/L]	Cadmium [micg/L]	Arsenic [micg/L]	Chromium [micg/L]
Iran [1] [n=88]	Mean	10.063	1.662	0.177	0.892	1.003
	Min	1.2	0.18	0.0	0.1	0.16
	Max	21.1	3.16	0.32	1.92	2.83
Malaysia [2] [n=13]	Mean	1.16	0.26	0.36	3.2	-
	Min	0.12	Tr	0.45	Tr	Tr
	Max	2.84	1.25	0.45	13.51	Tr
Turkey [3] [n=67]	Mean	3.01	0.21	0.37	1.77	0.64
	Min	0.9	0.21	0.29	0.12	0.14
	Max	14.2	0.32	1.36	30.63	6.4
Italy [4] [n=371]	Mean	5.51	350	0.38	3.5	1.1
	Min	Tr	Tr	Tr	Tr	Tr
	Max	47.49	3500	2	7	2
Germany [5] [n=132]	Mean	-	0.009	0.008	4.10	0.082
	Min	-	0.001	0.0006	3.20	0.006
	Max	-	0.76	0.265	5.00	1.72
Canada [6] [n=40]	Mean	0.65	5.3	0.2	-	-
	Min	<0.01	<0.1	<0.1	-	-
	Max	4.1	17.8	1.1	-	-
Serbia [7] [n=10]	Mean	-	0.34	0.06	0.65	0.346
	Min	-	<0.2	<0.01	<0.21	<0.04
	Max	-	6.32	0.18	1.51	1.06
Chile [8] [n=10]	Mean	7.46	-	-	6.15	-
	Min	0.25	-	-	<0.06	-
	Max	44.26	-	-	18.97	-
Bulgaria [9] [n=25]	Mean	-	-	0.011	3.08	0.86
	Min	-	<0.002	<0.001	0.29	<0.004
	Max	-	0.011	0.033	15.3	4.80

1: The current study. 2: Azlan et al. study in Malasya [31], 3: Guler et al. study in Turkey [32], 4: Naddeo et al. study in Italy [37], 5: Krachler et al. study in Germany [27], 6: Pip E. et al. in Canada [5], 7: Ristic et al. study in Serbia [30], 8: Daniele et. al, study in Chile [36], 9: Lyobomirova et. al, study in Bulgaria [1].

samples, which were exceeding the WHO guidelines [5, 38]. Gautam conducted a study investigating the chemical and trace elements in bottled waters. A total of 100 samples were analyzed for cadmium and lead levels using Flame Atomic Absorption Spectroscopy [FAAS], and both elements were not detected [39].

Al Aamri et al. examined the chemical composition of bottled drinking water in Oman. All analyzed brands had nitrate [NO3] levels lower than the US EPA [10 mg/L] and WHO maximum limits [50 mg/L]. Notably, four brands did not list their NO3 concentrations on the labels [40].

In a study by Bertoldi et al., the chemical composition of 571 European mineral water bottles was analyzed [41]. The overall mean aluminum level in the experiments was 5.78 mg/L, with only four samples exceeding the maximum level of 147 mg/L. Cadmium was detected in 2.5% of samples, reaching a maximum level of 0.69 mg/L in an Italian sample, far below the legally mandated limit of 3 mg/L set by the European Community. Lead levels were consistently below the EU limit for mineral water, with the highest concentration of 0.44 mg/L found in an Austrian sample.

Lyubomirova et al. conducted another study examining 17 Bulgarian and eight imported mineral water brands purchased from the commercial Bulgarian network [1]. Aluminum [Al] was detected in all investigated water brands, ranging from 0.31 to 44.3 µg/L, which is well below the threshold limits. One imported water brand [Borjomi] had a value below the limit of detection [LOD], while the remaining waters had concentrations ranging from 0.26 to 1.35 µg/L. Lead [Pb] was below the LOD in all Bulgarian and imported waters, except for Pirin Spring water, which had a value of 0.011 µg/L, well below the threshold values. Cadmium [Cd] was below the LOD in 41% of the investigated Bulgarian waters, and the rest had values below admissible concentrations. Chromium [Cr] levels in Bulgarian waters were below admissible limits, but the concentrations in two Georgian waters exceeded WHO limits, though they were below the EPA maximum concentration level.

A similar Iranian survey was conducted on 42 brands of bottled mineral and drinking, collected during two-year period from 2010 to 2013, revealed concentrations of lead, cadmium, copper, arsenic, and mercury in the order of4.50 ± 0.49, 1.08 ± 0.09, 16.11 ± 2.77, 5.80 ± 1.63 and 0.52 ± 0.03 µg/L. All the measured components were within the permissible values determined by international standards, which were in consistent with our study [42]. In another Iranian study by Salehi et al. 2012, nitrate, nitrite, and pH were shown to be of the normal range (being in consistent with our study) in total of 33 purchased bottled water produced in the Hamadan province of Iran (8.34 mg/L, 0.024 mg/L and 8.34, respectively) [43]. In the present study, mercury concentrations in 9 out of 11 samples were in excess of Australian Guidelines, which might be due to environmental water pollutions. Also, depicted constituent values on the labels in Pip E study occurred to be of different values regarding the analytical consequences in some of the samples [5]. These discrepancies may be contributed to changes during transferring these products towards the consumers. However, the origin source may have also affected the labeled quantities. Besides, metals components, which could be of low-level constituents in the water samples were not presented on the labels data at the time of purchasing the water samples. In the present study, we also had this discrepancy in all the samples particularly for nitrate and pH level, excluding 3 brands, which were similar to the labelling data.

CONCLUSION

Since the bottled drinking water of Iran are consumed not only in this country, and are exported to Iran's neighborhood countries such as Afghanistan, Pakistan, and Tajikistan, the water trade and health authorities of Iran should consider the heath safety of the water. It is required to find out the sources of mercury elevations [>1 µg/L] of nine out of eleven samples. It is also important that authorities find out why nearly all the analyzed constituents of the selected brands including nitrate and pH had significant discrepancy, compared to the depicted data of bottled water samples. Since the bottled water is of the ubiquitous sources for daily water consumption in large cities, it is of crucial importance to evaluate the quality of mineral composition and prevent further adverse effects of toxic heavy metals, nitrate, and nitrite. Hence, the authorities should consider the need of regular assessments of bottled drinking water qualities.

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